RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 1-28 and 30 were pending at the time of the Action. Claim 29 was withdrawn following restriction.

Claims 1 and 15 are amended to clarify that the selection temperature is from 30°C to about 34°C. Support is found at paragraphs [0019] and [0100] of the specification, in Table 3, and in the claims as originally filed. Claims 3 and 17 are canceled without prejudice in view of the amendment of claims 1 and 15. Claims 4, 5, 18, 19, 23, 25, 27, and 28 are amended in view of the amendments to claims 1 and 15, and in order to more clearly claim the Applicants' invention. No new matter is added.

B. Rejection of Claims Under 35 U.S.C. §112, First Paragraph - Enablement

The Action rejects claims 1-27 as lacking enablement. In particular, the Action asserts that the specification does not reasonably provide enablement for all transformation methods, all cereal plants and all types of explants. Applicants respectfully traverse.

With respect to enablement of transformation methods, Applicants note that the claimed method relates to selection of plant tissues after transformation is performed by a chosen method. This is recited in the claims, for instance claim 1, in particular in step 2. Numerous methods for transformation of plants, including cereal plants, are well known in the art, and are described in the specification. The claims relate to the surprising finding that selection of particular culture conditions, after contact of the plant tissues with exogenous DNA (*e.g.* via co-culture with *Agrobacterium,* or particle bombardment), improves the ability to obtain transformants from the plant tissue. The precise method chosen for transformation is irrelevant to the enablement of the

claims, because the culturing of step 2 takes place after transgene insertion. All that is relevant for purposes of enablement is that a tissue can be obtained for culturing comprising an introduced nucleic acid, which is clearly the case.

The Action maintains that both the transformation method and the explant are relevant to enablement, apparently because the effect of increased temperature on transformation efficiency, during selection is alleged to not be well known in the art. Applicants again note that this effect is found to occur after contact of an explant with exogenous DNA. In contrast, the temperatures typically used during the step of contacting an explant with exogenous DNA, *i.e.* while the given transformation method is being applied, are quite similar, and are less than the claimed selection temperatures, for instance as shown in scientific publications relating to transformation in cereal plants that Applicants submitted as evidence in the previous response, as well as publications cited by the Examiner. Analysis of these publications below demonstrates, for instance, (1) that successful transformation occurred via several methods; (2) that numerous explant tissue types were utilized in the different cereal species; (3) that transformation was performed at various generally overlapping temperatures ranging between 20-27°C; and (4) that co-cultivation (if appropriate) and selection was also performed at these same temperatures:

a. S. Tingay *et al.*, *Agrobacterium tumefaciens*-mediated Barley Transformation (*Plant J.* 11:1369-1376, 1997). Explants used were immature embryos ("IE's") injured by bombardment and grown on callus-inducing medium. Thus the explant subjected to selection was apparently a mixture of embryonic cells and (induced) callus cells. Transformation was via an *Agrobacterium*-mediated method; co-cultured and selected at 24°C.

b. I. Roussy *et al.*, Transformation and Regeneration Capacities for Five Nordic Barley Elite Cultivars – Evaluation of Tissue Culture Response and Transient Expression (*Hereditas* 134:97-101, 2001). Stated to use the method of Tingay (above); also

performed particle bombardments, although only transient transformation results described in that instance.

c. Y. Hiei *et al.*, Efficient Transformation of Rice (*Oryza Sativa* L.) Mediated by *Agrobacterium* and Sequence Analysis of the Boundaries of the T-DNA (*Plant J.* 6:271-282, 1994). Numerous explant tissues utilized, including: IE's, scutellum-derived callus; suspension-cultured callus; root-derived callus; roots; shoot apices. Transformation via an *Agrobacterium* mediated method; co-culture and selection at 25°C.

d. M. Valdez *et al.*, Transgenic Central American, West African and Asian Elite Rice Varieties Resulting from Particle Bombardment of Foreign DNA into Mature Seed-derived Explants Utilizing Three Different Bombardment Devices, (*Annals of Botany* 82:795-801, 1998). Used bombardment of IE's, selected on callus induction medium at 27°C. Thus explant being selected was apparently a mixture of embryonic and (induced) callus cells.

e. M. Cheng *et al.*, Genetic Transformation of Wheat Mediated by *Agrobacterium tumefaciens* (*Plant Physiol.* 115:971-980, 1997). Explant tissue was IE's and embryogenic callus. Transformation was via an *Agrobacterium* mediated method; inoculation at 23-25°C; co-culture and selection at 24-26°C.

f. A.M. Casas *et al.*, Transgenic Sorgham Plants via Microprojectile Bombardment (*Proc. Natl. Acad. Sci.*, USA, 90: 11212-11216, 1993). Used particle bombardment of IE's placed on callus induction medium; thus explant being selected was apparently a mixture of embryonic and callus tissue. No temperatures explicitly given in reference; presumably room temperature, *i.e.* within the range of temperatures given in the other references.

g. K.A. Torbert *et al.*, Transformation of Oat Using Mature Embryo-Derived Tissue Cultures (*Crop Sci.* 38:226-231, 1998). Transformation was via particle bombardment of embryos and callus tissue. Growth and selection of tissue at 20°C.

h. R.V. Sairam *et al.*, Shoot Meristem: An Ideal Explant for *Zea mays* L. Transformation (*Genome* 46:323-329, 2003). Explant tissue was shoot meristems; transformation was via an *Agrobacterium* mediated method; explants were placed on callus induction medium, thus the tissue being selected was apparently a mixture of meristematic and callus tissues. Temperatures are not explicitly given and are apparently room temperature, although U.S. Patent Application Publication 20040237133, apparently describing the same or similar work, describes culture of some tissues at 20-24°C.

Thus, Applicants note again that multiple tissue explants from numerous cereals including immature embryos, callus derived from immature embryos, suspension-cultured callus, shoot apices, and shoot meristems, among others, have been used with several methods including *Agrobacterium*-mediated, particle bombardment, and PEG-mediated transformation. In each case, the temperatures used during transformation and selection were in the range of 20-27°C, well below the claimed temperatures. In some instances the references do not even describe the temperatures used during selection of transformed tissue, which Applicants submit is an indication that the temperature during selection was not considered an important variable, contrary to the present disclosure. Temperatures used for selection of the various cereal tissues were largely overlapping, either when different transformation methods or explant tissue types are compared within the same species, or when the same transformation methods and/or explant types are compared between different species. There is, therefore, no basis in the cited or submitted references for the allegation that the different explants have different preferred temperatures for selection, or that explants would show different sensitivity to elevated temperature during selection, depending on the transformation method used (Action, page 4).

Although the Action notes that the temperature described for oat tissues by the Torbert reference is lower (20°C) than that, for instance, described for maize, Applicants note first that

this assertion does not in any event properly relate to claims 1-14, which explicitly recite maize tissue. Second, the work of Sairam described above also includes culture of maize tissue at 20-24°C, as does the cited work of Frame *et al.*. Third, although Applicants do not concede that the allegation is correct, to advance prosecution claim 15 is amended to recite wheat, rice, barley, and sorghum plants.

Simply put, routine methods for transformation of various explant tissues from numerous cereals were well known, and may easily be applied by one of skill in the art, to obtain explant tissues that have been contacted with and putatively transformed by exogenous DNA. The present invention does not relate only to these steps in the process of obtaining transformed tissues and plants. Instead, the present invention uses such putatively transformed explants (*e.g.* step 1 of claims 1 or 15) and relates to efficiently selecting successfully transformed explants and resulting tissues (*e.g.* step 2 of claims 1 or 15), including via use of an elevated temperature during the selection process (specification, paragraph 0100). The knowledge in the art coupled with the descriptions and examples in the current specification thus fully enables the claims. While some experimentation may be required, any experimentation would be routine given the substantial teaching in the specification and the knowledge in the art. In sum, Applicants have affirmatively demonstrated enablement of the claims and no basis to doubt the enablement has been provided. Removal of the rejection is thus respectfully requested.

D. Rejection Under 35 U.S.C. §102(b)

The Action rejects claims 1, 3, 5-10, 14-17, 19-24, 28 and 30 as anticipated or obvious over Frame *et al.* in view of Zhao *et al.* (*Molecular Breeding* 8:323-333, 2001). In particular, it is asserted that the references teach that selection of transformed cells is carried out at 28 °C,

which is interpreted to include "about 30°C". Applicants respectfully traverse, while noting that claims 1 and 15 have been amended to recite "30°C to about 34°C".

Given the general knowledge in the field of plant cell culture, as well as the present disclosure, that temperature may be an important variable in plant cell culture (although not during selection following transformation, in the prior art), one of skill in the art, and in accordance with the teachings of the present application, would not simply assume that "28°C" is equivalent to "about 30°C". In particular, Table 3 of the present application describes a set of experiments wherein temperature changes of 2°C, *i.e.* 30°C, 32°C, and 34°C, are compared among themselves and with a treatment of 27°C for their effect in selecting transformed tissue. Thus, the present application clearly indicates that a 2°C change in the selection temperature represented a change that was large enough to warrant quantification of possible effects. The Action does not assert that the cited reference teaches or suggests temperatures above 28°C, and simply asserts, without any basis, that these temperatures (28°C and "about 30°C") are equivalent during the selection process.

In view of the above, the cited references do not teach all elements of the current claims and cannot form the basis for an anticipation rejection. However, to advance prosecution, the claims have been amended to recite "30°C". Applicants respectfully submit that 28°C and 30°C are not equivalent temperatures. The rejection is therefore believed moot and removal thereof is respectfully requested.

E. Rejection Under 35 U.S.C. §103

The Action rejects claims 1-10, 14-24, 28, and 30 as obvious over Frame *et al.* in view of Zhao *et al.* In particular, it is asserted that different times of selection and different selection

temperatures involve optimization of process parameters, and do not represent a patentably distinct invention, given that 28°C is interpreted to mean "about 30°C". Applicants respectfully traverse, and also note that claims 1 and 15 have been amended to recite "30°C".

Thus, Applicants note that the Action concedes that the cited art is lacking any suggestion or motivation to select transformed cells at higher than 28°C, or in the temperature range defined by the present claims. At the time of filing the current application, selection temperature was not regarded as an important parameter for investigation in studies of transformation efficiency. There is therefore no basis for an obviousness rejection. Specifically, as noted previously, nothing in the cited art suggests that benefit could be obtained by increasing selection temperatures or in particular by use of selection at 30°C or higher. Selection temperature is not a parameter that would routinely be varied, absent the present disclosure. In contrast, Applicants have shown that such conditions may be used to achieve increased transformation frequency as seen in Example 8 and Table 3 of the specification.

With selection temperature considered unimportant, the prior art provides no reason to vary selection temperature or to try to optimize the selection temperature. As such, the prior art provides no motivation to select cells at higher temperatures. Moreover, the increase in transformation frequency upon selection at higher temperatures is conceded to be unexpected. In particular, the Action at page 6 states "It may be true that the increase in transformation frequency upon selection at high temperature is unexpected…" Applicants respectfully submit that the invention can not simultaneously be both obvious and unexpected. In view of this and of the amendment of claims 1 and 15 to recite "30°C", the claims are not properly rejected as obvious under §103 and removal of the rejection is thus respectfully requested.

CONCLUSION

In view of the foregoing, Applicants respectfully request favorable consideration of this case.

The Examiner is invited to contact the undersigned attorney at (214) 259-0931 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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